ADAPTATION OF THE MAGNETORECEPTIVE MECHANISM OF MUD–SNAILS TO GEOMAGNETIC STRENGTH [1]

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The mud-snail, *Nassarius,* has been proven to be sensitive to a magnetic field as weak as 1.5 gauss and to be able to distinguish between fields parallel and at 90° to its path of locomotion, (Brown, Brett, Bennett and Barnwell, 1960; Brown, Webb and Brett, 1960; Brown, Bennett and Webb, 1960). A similar capacity has been reported for *Volvox* by Palmer (1963), who employed 5-gauss fields. An extensive study by Brown (1962) of the planarian, *Dugesia,* established that this worm, too, displayed extraordinary sensitivity to very weak magnetic fields. It was demonstrated that in an unchanging illumination field, the tendency of the worms to veer from an initial direction of movement varied in a manner characteristic of each compass direction of the initial path. The rotation of a 5-gauss magnetic field had qualitatively the same effect on the path taken as did equivalent reorientation of the worms themselves in the earth's field. Qualitatively the same type of response was also evident for the weaker field strengths, 2.0 and 0.25 gauss, but the response became progressively weaker as the experimental field strength approached that of the earth's own horizontal field, 0.17 gauss, which was always present. However, when the field strength was raised to 10 gauss, the normal response disappeared and was replaced by an opposite, or mirror-imaged, compass directional pattern. Upon the basis of preliminary experiments it has also been reported that changing the orientation of a 5-gauss horizontal field gives a response pattern which is the inversion of that observed upon changing the orientation of snails in the earth's field (Barnwell and Brown, 1964).

While the foregoing experiments strongly suggested that the receptor system for organismic response to the horizontal vector of magnetism was one specialized for such weak fields as the earth's, the method of study would not permit resolution of the particular field strength for optimal response. In consequence, the experiments to be reported here were designed and conducted. The results point strongly to the conclusion that the optimal resolving capacity of a living system for the direction of the horizontal component of magnetism is exquisitely adjusted to the strength of geomagnetism.

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Methods and Materials

The mud-snails, *Nassarius obsoletus*, which were used throughout this study were collected once or twice a week at Chapoquoit beach on Buzzards Bay, a few miles north of Woods Hole, Massachusetts.

The experiments consisted of assaying the turning tendency of the snails moving initially magnetic south in the earth's field and alternating these assays with measurements of the turning tendency of the same snails subjected to an abruptly reversed experimental field. The reversed field was produced by a cylindrical bar magnet placed horizontally beneath the organisms and oriented to oppose the earth's horizontal vector and override it to produce a series of strengths of the reversed field as follows: 0.04, 0.1, 0.2, 0.4, 0.8, 2.0, 5.0 and 10.0 gauss. The experiments were performed over two-month periods during the summers of 1960, 1961 and 1962, with slight differences in the procedure between one summer and the next. In each year, the experiment was performed in quadruplicate, with four observers working concurrently with four identically constructed pieces of apparatus.

The apparatus has been described in some detail elsewhere (Brown, Brett, Bennett and Barnwell, 1960; Barnwell and Brown, 1964).

Experiment I was performed in 1960 during the two-month period from June 29 to August 26, inclusive. Quadruplicate series were run on 47 mornings, between 9:30 and 12 o'clock, distributed as uniformly as was practical over the whole two-month period. For purposes of analysis, the total period was subdivided into two monthly periods. Month 1 included 22 days of observation from June 29 through July 26, and month 2 comprised 25 days of data obtained between July 28 and August 26, inclusive. The snails emerged from the exit of an aluminum, funnel-shaped corral into a symmetrically illuminated field with white backgrounds to both right and left.

Each experimental series consisted of 16 sets of 5 snail paths; a set of 5 paths obtained in the earth's field regularly alternated with a set of 5 paths obtained in a reversed horizontal magnetic field. Eight different field strengths were tested, always in the same order. The pattern of each experimental series was as follows: C (= control); 0.04 (gauss); C; 0.1; C; 0.2; C; etc. Immediately upon completion of these observations the entire series was repeated but in reversed order. Thus, each of the four observers on each morning assayed a total of 10 snail-paths under each of the 16 conditions.

Experiment II was carried out during the summer of 1961. The experiments were conducted, again in quadruplicate, on 26 mornings between 9:30 and 12 o'clock, usually on three alternate mornings a week, between June 22 and August 19, inclusive. For analysis the data were subdivided into month 1, including 13 days between June 22 and July 20, inclusive, and month 2, consisting of 13 days from July 22 through August 19. In these experiments the snails emerging from their corral entered an asymmetrical field of illumination with black to left and white to right. The snails, in general positively phototactic, tended to veer to the right. Each daily series comprised observing the paths of 5 snails emerging under each of 14 conditions; then 5 additional paths were observed under each of the conditions presented in reversed order. The 14 conditions in their initial order were: C; 0.04; 0.1; C; 0.2; 0.4; C; 0.8; 2.0; C; 5.0; 10.0; C; and C with a reversed asymmetrical field. Hence, each observer obtained the paths of ten snails under each of the 14 conditions.

Experiment III was performed on 18 afternoons between 1 and 3:30 o'clock, usually on Tuesday and Thursday each week over the period, June 21 through August 20, 1962. Month 1 comprised 9 days between June 21 and July 19, inclusive, and month 2 included 9 days from July 24 through August 20. In this experiment the snails emerged into a symmetrical field with large aluminum plates to right and left. The plates were interconnected to assure their equipotential condition but were not grounded. The mean paths for 5-snail samples were determined by each of two observers for each of 13 conditions in the following order and then, at once, for the same series in reversed order: C; 0.04; 0.1; C; 0.2; 0.4; C; 0.8; 2.0; C; 5.0; 10.0; and C. At the same time two other observers were proceeding through comparable series which differed only in that the successive pairs of the magnetic fields were reversed (*e.g.*, C; 0.1; 0.04; C; 0.4; 0.2; C; 2.0; 0.8; . . .).

In experiment I, conducted in 1960, each of the sectors of the polar coordinate grid from which the mean snail paths were determined at 22.5°. For experiments II and III during the succeeding two summers, the sectors were reduced in size to 11.25°.

In view of the fact that the response of snails to weak magnetic fields has been demonstrated to display solar-day (Brown, Brett, Bennett and Barnwell, 1960) and lunar-day (Brown, Webb and Brett, 1960) variations and consequent monthly or semi-monthly ones, the data were reduced at once to monthly values. For each of three years there were available four individually obtained series for each of two months—a total of 24 monthly values representing six different months. The influence on the turning tendency of the snails of each of the eight experimental field strengths was computed separately for each of the 24 series of values. Two methods of computation (Methods A and B below) were carried out for each series.

By method A for the 1960 experiment I, the mean path in each strength of reversed magnetic field was expressed as the difference from the adjacent control sample which preceded it as the series was run in one direction and followed it when the order was reversed. By method B the mean path of the snails for each strength of reversed field was expressed as the difference from the mean path obtained by averaging all controls.

Methods A and B for computing the influence of the experimental fields were applied in a comparable manner to the data of the succeeding two summers. For experiment II, in 1961, method A consisted of expressing the effects in terms of differences from the immediately adjacent controls. As a consequence, the members of the pairs of strengths, 0.1 and 0.2, 0.4 and 0.8, and 2.0 and 5.0 gauss, had common controls. Method B consisted of expressing the influences of the experimental fields in terms of differences in paths from the average path for all controls. For experiment III, in 1962, method A consisted of expressing the mean paths of the snails in each experimental field strength as the difference from the mean path in the two control samples flanking it, both before and after, as the series was run first in one order and then the reversed order. Now, two common controls were shared between 0.04 and 0.1, 0.2 and 0.4, 0.8 and 2.0, and 5.0 and 10.0 gauss, and successive pairs shared one control. Method B in 1962 consisted of expressing the mean path at each strength of reversed field as the difference from the average path for all controls.

The two methods of analyzing the influences of the various strengths of the experimentally reversed fields were employed inasmuch as nothing was initially known concerning whether there were any persistent effects following exposure of the snails to the various experimental fields. It must be noted that as far as magnetism is concerned, two kinds of abrupt reversals were being imposed upon the snails. One of these was the reversal at each of a series of strengths of field as the animals were changed from the earth's 0.17-gauss horizontal field to a lower or higher reversed one, and the other was the return of the snails from the lower or higher reversed experimental field back to the earth's natural one.

It was possible that a persisting influence of abrupt field reversal, or abrupt changes in field strength, or even in some combinations of these changes might occur. If such an influence manifested itself as a modification of the path taken by the snails in subsequent tests, the use of adjacent controls to calculate the effects of experimental conditions might give misleading results. Depending on the direction in which the path was modified the results might indicate effects either larger or smaller than the true ones. In view of this second kind of problem, one additional type of analysis was made. This involved study of the mean paths of the snails in the earth's south-directed 0.17 gauss field, employing the data of the controls in the series of experiments I, II, and III. The mean path for each control sample was considered in relation to the average strength of the experimentally reversed fields which immediately preceded it.

This last consideration obviously required the elimination from this analysis of the first five control paths for all three years, inasmuch as these were preceded by no experimentally reversed field. It also required the elimination, for the same reason, of the first five control paths, as the series was commenced in the reversed order in experiments II and III in 1961 and 1962. The time to obtain a sample of 5 snail paths ranged typically from three to five minutes, and hence in this study the effect of an exposure of this duration to an experimentally reversed field was being assayed over an equal period of time immediately following the removal of the experimental field.

Results

Response to reversed fields expressed as difference from adjacent controls: Method A

In Figure 1A the response is plotted as a function of field strength for each of the three years. Response is calculated as the average deviation from the adjacent controls (Method A). Each point represents the average of all tests in a given year. Inspection of Figure 1A suggests a general over-all trend in the deviations in paths from the controls in passing from the weakest reversed field, 0.04 gauss, to the strongest, 10 gauss. Suggested is an apparent tendency toward right-turning in response to reversed fields weaker than the earth's and left-turning in response to reversed fields stronger than the earth's. This is based, of course, on the assumption that all the controls are fully comparable to one another and that there is no persistent influence of the experimental fields.

Of greater statistical significance, however, are the differences among the variances for the eight field strengths for the three-year study. Variance is maximum for the 0.2-gauss field and minimum for the 0.8-gauss one. Employing Hartley's (1943) method of largest F ratio to determine the probability that there is heterogeneity of variance among the eight samples, *F* max. was found to be 1097 (*P* <

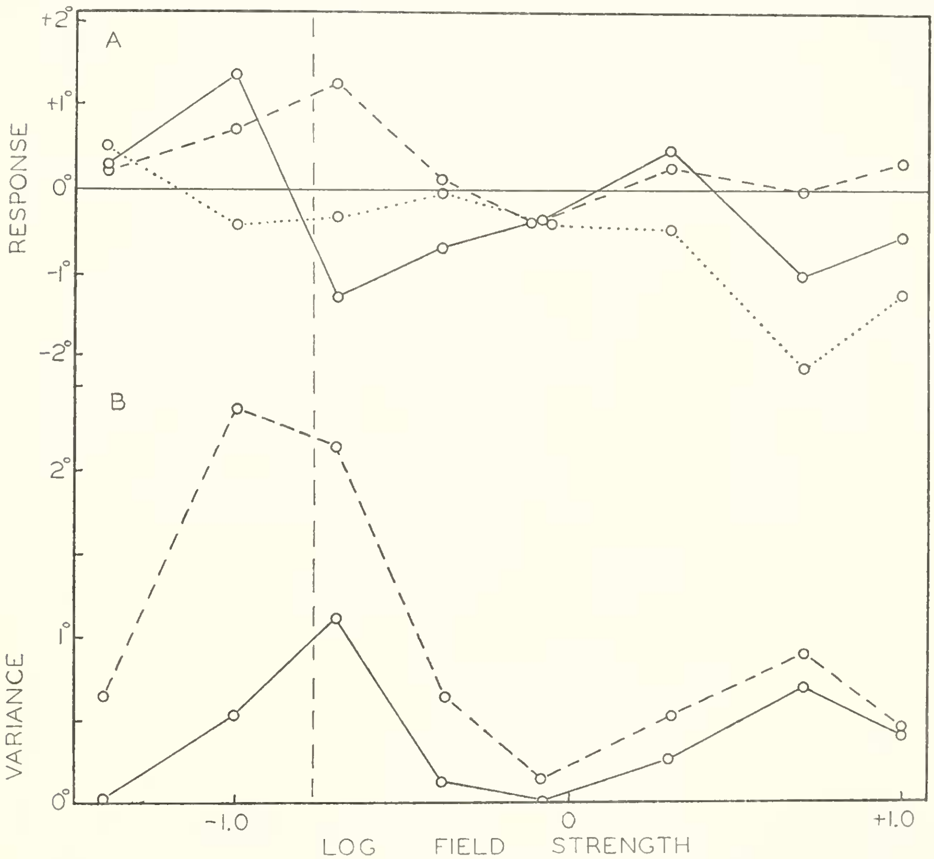

FIGURE 1. A. The mean deviation in snail path from that of adjacent controls in the experimental series, in response to experimentally reversed magnetic fields at a series of strengths. Solid line, 1960; broken line, 1961; dotted line, 1962. B. The variances obtained for each field strength computed from the three mean annual results (solid line) and from the 6 mean monthly ones (broken line). The vertical dashed line indicates the strength of the earth's horizontal vector.

0.05). In Figure 1B are plotted the variances of these mean responses for the eight strengths of reversed field. Included also in Figure 1B is the distribution of variances computed using all 6 monthly mean values obtained over the three years. In both of these instances the maximum tendency of the snails to deviate from the southward path in the earth's 0.17-gauss field in response to abrupt reversal of the horizontal vector of magnetism occurs for experimental field strengths closest to that of the natural terrestrial one.

A persisting influence of experimentally reversed fields on succeeding controls

The examination of the controls was next undertaken. In Figure 2 the mean values of controls for each of the three years are plotted in relation to the log of the mean experimental magnetic field strengths that immediately preceded them.

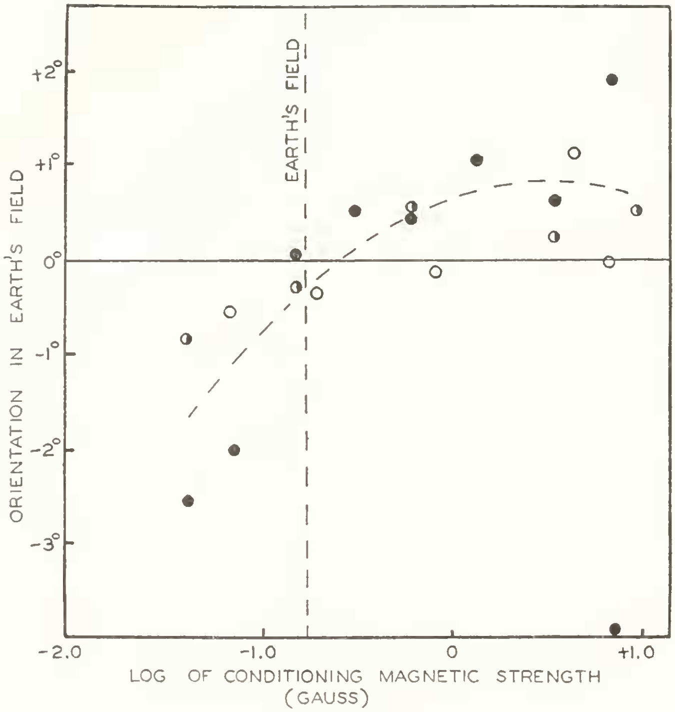

FIGURE 2. The relationship between snail path when southbound in the earth's field and the mean strength of the immediately preceding, experimentally reversed magnetic field. Broken line curve is the computed non-linear relationship. Solid dots, 1960; half-solid dots, 1961; circles, 1962. The values are expressed as differences from the average ones for all strengths for that year.

The controls for each of the three years are expressed as deviations from mean control for that year. It is evident from this figure that the paths of control snails in the earth's field are generally further to the left than the mean following subjection to a reversed field weaker than the earth's and further to right than the mean following subjection to a reversed experimental field stronger than the earth's. Treated as a linear correlation, $r = 0.75$ $N = 18$; $P < 0.001$.

This highly significant correlation indicates that the altered magnetic field exerted an effect that persisted for the three- to five-minute control assay after completion of the test exposure.

When the data depicted in Figure 2 were treated as a non-linear correlation, a better fit was described by the equation

$$Y = 0.631 + 0.695X - 0.696X^2,$$

where X is the log of the field strength expressed in gauss. This relationship is described by the broken line in Figure 2. The non-linear coefficient of correlation was 0.825, indicating that more than 68% of the variation was described by this relationship.

The specific character and magnitude of the magnetic effect persisting in the control snails was more than enough to account for the apparent over-all gradual increase in left-turning in response to increasing strengths of reversed field (Fig. 1A) when these were computed as differences between paths in the reversed fields and paths in adjacent controls.

Response to reversed fields expressed as difference from the mean of all controls: Method B

This method for dealing with the data eliminates any influence of differences among the controls and permits a comparison among the mean paths in the reversed fields of 8 strengths.

In Figure 3A are plotted the relative effects of the eight reversed fields for each of the two months of 1960. The results for the two months display a general similarity to one another. Maximum turning, to the left, for the summer occurs in response to the reversed 0.2-gauss field.

The results obtained for each of the two months of 1961 are illustrated in Figure 3B. Again the results for the two months show a good resemblance to one another ($r = 0.713$); a maximum in turning again occurs at 0.2 gauss, but the direction is opposite that obtained for 1960.

In the experiment performed in 1962, the results obtained for the two months showed a striking difference between them. The results for the two months, for fields weaker than 2 gauss, appeared to mirror-image one another. The results for the first month were strikingly similar to the average of those obtained for the summer of 1960 ($r = 0.805$). The apparent response to the 0.2-gauss field was $-2.25 \pm 0.29°$ ($N = 4$). The results obtained for the second month were more similar to those obtained for 1961.

In Figure 3D are seen the average results for each of the three years, together with selected standard errors. It is evident that the most significant deviation in path from the controls for both 1960 and 1961 occurs at 0.2 gauss with values of $-1.738 \pm 0.526°$ ($N = 8$) and $+0.882 \pm 0.250°$ ($N = 8$), respectively. At this strength of magnetic field were also the most consistent responses, as indicated by the lowest variance in the series of eight field strengths for each of these two years. However, for 1962, as would be expected from inspection of Figure 3C, the response to the 0.2-gauss field exhibits the maximum variance in the series of strengths. The mean path for both months was $-0.750 \pm 0.829°$. The F max. for the 8 individually-obtained series of 1962 was found to be 34.25, indicating a significant heterogeneity of variances among the eight field strengths ($P < 0.01$), with the largest variance at 0.2 gauss and the smallest one at 2 gauss. These results appeared consistent with the results of 1960 and 1961 in showing maximum response to the 0.2-gauss field, but the response, as in these other years together, was either clockwise or counterclockwise turning.

In Figure 4, the relationship between variance of mean paths and strength of reversed field is shown for the 6 months of series of responses for which response

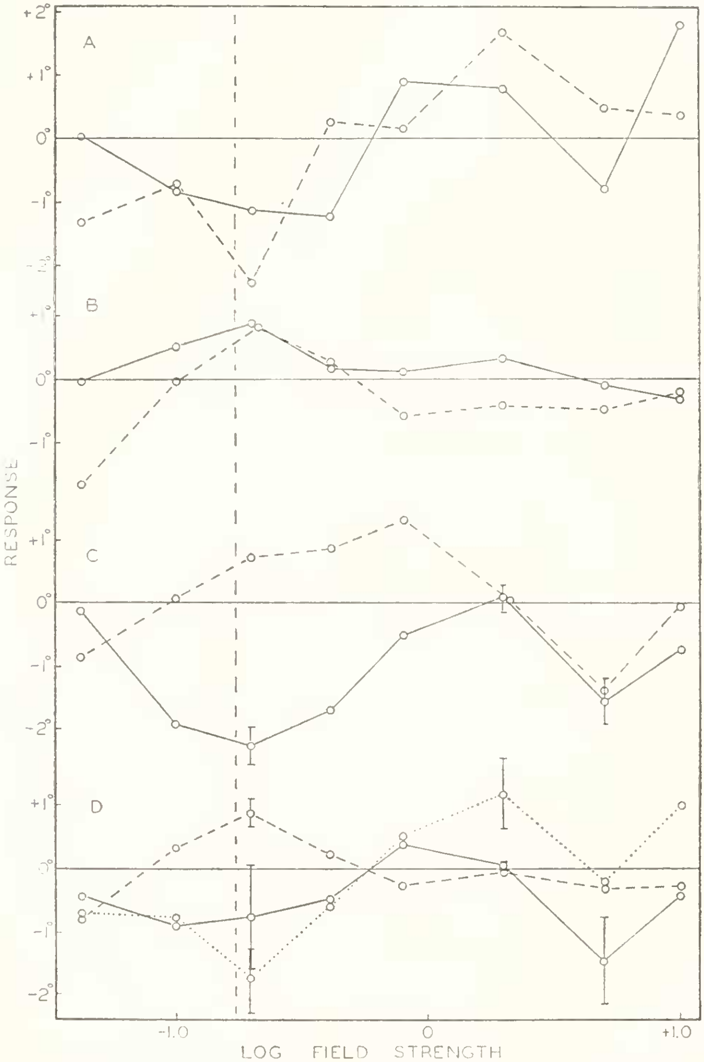

FIGURE 3. The deviation in snail path from the average path for all controls in the series for each of a series of eight strengths of experimentally reversed horizontal vectors of magnetic field. A. Month 1 (solid line) and month 2 (broken line) in 1960. B. Month 1 (solid line) and month 2 (broken line) in 1961. C. Month 1 (solid line) and month 2 (broken line) in 1962. D. The mean annual results for 1960 (dotted line), for 1961 (dashed line), and for 1962 (solid line) expressed as deviations from mean response. Selected standard errors are shown. The vertical dashed line marks the strength of the earth's horizontal component.

was determined as deviation from the average path for all controls in the series (method B). Maximum variance again occurs at 0.2 gauss with a value of 2.014°. Minimum variance, 0.237°, occurs at 0.8 gauss. The ratio of these variances, or F, is 8.5. ($P < 0.01$)

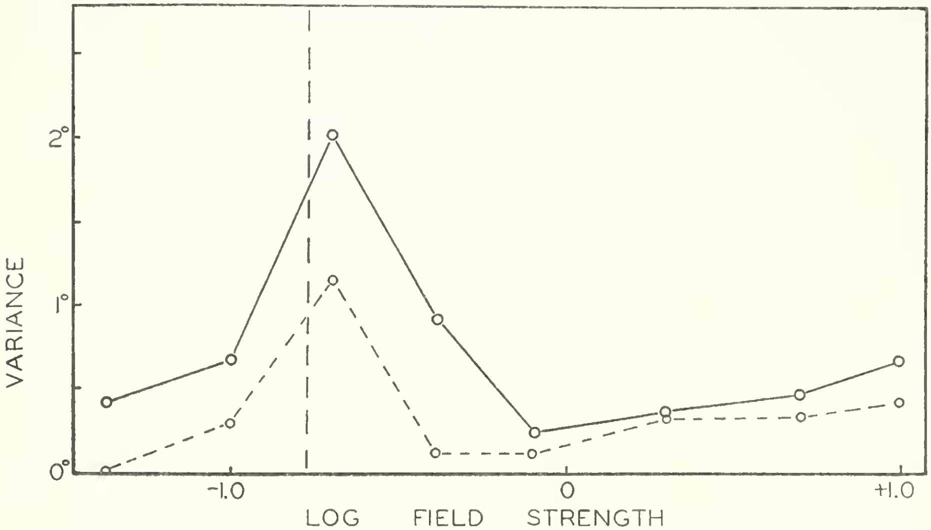

FIGURE 4. The variances obtained for responses to the reversed fields as computed from the data illustrated in Figure 3. The solid curve shows variances computed from 6 mean monthly values, and the broken curve, from three mean annual ones. The vertical dashed line indicates the strength of the earth's horizontal component.

DISCUSSION

Mud-snails initially directed geographic southward display a characteristic mean turning tendency away from this southward path; when directed geographic north, a different mean turning tendency is evident (Brown, Webb and Barnwell, 1964). It was the intent of this investigation to learn at what strength of an abruptly reversed horizontal magnetic vector the snails would be induced to alter to the greatest degree their characteristic "south"-turning tendency.

It is significant of itself that despite the fact that the conditions of the experiment differed over the three summers of study in symmetry of background, in state of the ambient electrostatic field, and in time of day, one relatively uniform result was found. This was that the maximum tendency to alter direction in response to the experimentally reversed horizontal magnetic field appeared to occur when the strength of the reversed field was that one closest in strength to the natural terrestrial one.

On the other hand, it is unfortunate that the variety of conditions used for experiments renders it impossible at this time to reach any similarly well supported conclusion as to why the response should have been for three out of the six months a counterclockwise response, with a clockwise one for the remaining months. That it probably involves the influence of some factor other than any of those involved in the controlled experimental conditions is indicated by the differences observed between the two months of 1962, despite the similarity of all controlled conditions.

The results as described in the relationship between the experimental field strength and the observed variances do, however, indicate a rather sharp adjustment of the receptor mechanism for magnetic compass direction to the strength of the horizontal vector of geomagnetism, H, rather than to, for example, the total earth's field strength, F, of about 0.5 gauss. It is evident that the responsiveness of the snails has already fallen off greatly even before the horizontal vector strength has risen to 0.4 gauss, and has become minimal at about 0.8 gauss.

It would be difficult to resolve the exact strength for maximum responsiveness to the horizontal vector of magnetism. This difficulty results in part from the variability in strengths and vector directions of these very weak fields that one encounters in and about the ordinary laboratory. Perhaps in far larger measure it results from the discovery, reported here for the first time, of persistent effects of magnetism. These were illustrated in Figure 2. From the figure it is evident that the strength, and possibly even the sign of the apparent response of the snails to terrestrial magnetism, can be altered by a brief conditioning exposure to a slightly stronger or weaker experimentally reversed horizontal field.

Additional and indirect support for the conclusion that maximum capacity to resolve the direction of the horizontal magnetic vector occurs when the simultaneous strength-change has been least comes from Figure 2. The change in sign of the mean response from negative to positive, relative to the mean response for all paths, occurs as the conditioning stimulus strength approaches, and passes through, the strength of the earth's weak horizontal vector.

The results shown in Figure 2 also emphasize the obvious caution needed in any interpretation of studies of magnetic response which may at some future time be carried out in magnetically shielded environments where the ambient field strength is greatly reduced. The response for magnetism appears to resemble other kinds of receptor systems in possessing an ability to adapt or adjust to changing levels. This seems to be true at least within the narrow range of strengths spanned in this investigation.

Such properties as those of the extraordinarily adjusted maximum sensitivity to the level of geomagnetism, even to the specific local horizontal vector strength, and the persistent induced alterations occurring in response to very small field-strength changes lead one to postulate that the mechanism that is concerned in this response is biologically adaptive, rather than a useless, fortuitous property of protoplasmic systems. It appears probable, therefore, that it plays important roles in the lives of the organisms.

Summary

1. The maximum orientational response of the snail, *Nassarius*, to an abrupt experimental reversal of the horizontal vector of geomagnetism occurs when the reversed field deviates least in strength from the earth's horizontal 0.17-gauss one.

2. The responsiveness to field-direction change drops off very substantially even before the field strength has decreased to 0.1 gauss or increased to 0.4 gauss. It is already minimal at 0.8 gauss.

3. The receptor mechanism for compass orientation in response to geographic direction is, therefore, exquisitely adjusted to the strength of the horizontal vector of geomagnetism, even in contrast with the total strength of geomagnetism.

4. There is a persistent effect of experimental magnetic fields deviating in strength from the natural one which remains for at least three to five minutes following removal of the experimental field.

5. Following exposure to reversed horizontal fields stronger than the earth's, southbound snails respond in the earth's field by clockwise turning, and following exposure to reversed fields weaker than the earth's, by counterclockwise turning, relative to their path in the earth's field following exposure to an experimentally reversed horizontal field of the strength of the earth's natural one.

6. These results suggest that the receptor mechanism of the organism for very weak magnetic fields is highly specialized and adaptive, probably playing important roles yet to be disclosed.

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